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Impact of weather on off-flavour episodes at a Louisiana commercial catfish farm

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Abstract

The catfish aquaculture industry is hampered by off-flavour events that affect timely fish sales. In this study, weather data were correlated with geosmin and 2-methylisoborneol (2-MIB) levels in 21 commercial pond's water samples. Samples were collected weekly for 44 weeks. The off-flavour compounds, geosmin and 2-MIB, were quantified using gas chromatography–mass spectrometry. Weather data were collected near the catfish farm and included maximum and minimum air temperature, rainfall, average wind velocity, maximum and minimum humidity, and maximum and minimum soil temperature. Geosmin was weakly and positively correlated with air and soil temperatures, and weakly and negatively correlated with wind velocity. 2-MIB was strongly and positively correlated with air and soil temperatures, moderately and negatively correlated with wind velocity, and weakly and positively correlated with maximum humidity. There were no bivariate relationships between rainfall, minimum humidity or pond size, and levels of either off-flavour compound. Using logistic regression, the best predictors for off-flavour status based on geosmin levels included minimum soil temperature, rainfall and minimum humidity. The best predictors for off-flavour status based on 2-MIB levels included minimum soil temperature and average wind velocity. Soil temperature and rainfall were risk factors for off-flavour, while humidity and wind velocity were protective factors.

Keywords: aquaculture, off-flavours, geosmin, 2-methylisoborneol

Introduction

Catfish aquaculture in USA in 2007 yielded nearly 500 million pounds of processed fish (US Department of Agriculture, NASS). Production is intensive, with large inputs of feed that result in dense cyanobacterial growth. Some cyanobacteria in the production ponds produce odorous secondary metabolites that are absorbed by the fish rendering them off-flavour and unmarketable (reviewed by Tucker 2000). Geosmin and 2-methylisoborneol (2-MIB) are the two most common of these compounds and have a characteristic muddy/earthy aroma. Consumers can detect geosmin and 2-MIB at very low concentrations (low ng L^{-1}). Further, geosmin and 2-MIB are hydrophobic compounds that accumulate in fish flesh to higher concentrations than is present in the pond water (Lelana 1987; Johnsen & Lloyd 1992; Grimm, pers. comm.). The relative abundance of off-flavour-producing cyanobacteria changes throughout the course of the year, with the highest off-flavour occurrence during summer and fall, although off-flavour episodes can occur at any time of the year (Lelana 1987; Tucker & van der Ploeg 1993; van der Ploeg & Tucker 1994; Zimba, Grimm, Dionigi & Weirich 2001; reviewed by Tucker 2000).

In this study, we examined environmental factors that co-occur and may influence off-flavour episodes.

A weather monitoring station operated by the Louisiana State University's Agriculture Center (see http://www.lsuagcenter.com/weather/) is proximal to a commercial catfish farm in southern Louisiana. This weather data afforded intensive assessment of causality as compared with previous short-term studies. Twenty-one ponds at this farm were sampled weekly for 44 weeks and tested for geosmin and 2-MIB using gas chromatography–mass spectrometry (GC–MS). These data were analysed using descriptive statistics, correlations and logistic regression. Results from the regression models indicate that certain specific weather conditions can be helpful in predicting off-flavour events.

Materials and methods

Sample collection

The Limco catfish farm is located northwest of Crowley, LA (30°17′48″N, 92°31′48″W) and consists of 37 ponds ranging from 2.1 to 6.3 ha (Fig. 1). Twenty-one of these ponds were sampled weekly between 10:00 and 12:00 hours from September 2002 to October 2003. Inclement weather sporadically prevented water sample collections. Figure 1 depicts the layout of the 21 ponds that are identified numerically. The stars indicate the position of a small dock where all water samples were collected for this study. Whereas these points are not uniformly positioned on one geographical side of the ponds, those were the only

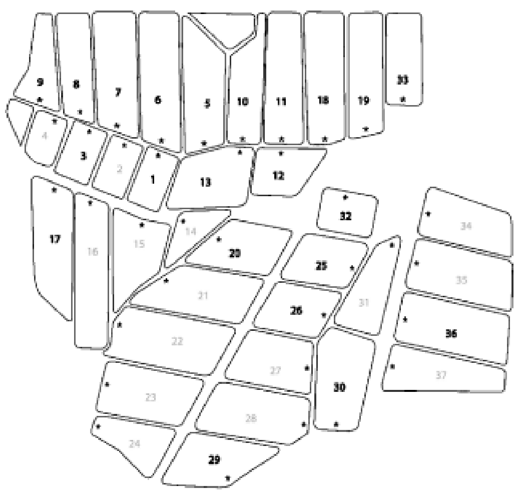


Figure 1 Schematic of the ponds at Limco catfish farm. All ponds are numbered. The numbers in bold font denote ponds used in this study.

available access points and allowed sufficiently deep samples to be taken. An integrated sampler (5 cm × 100 cm) was used to collect 2 L of pond water. Fifty-millilitre sub-samples were removed to disposable polypropylene tubes and stored on ice. The remaining water was stored in 2 L polypropylene bottles on ice. Samples were held until approximately 09:00 hours the next day, at which time 6 mL samples were transferred to autosampler vials for geosmin and 2-MIB analysis. It should be noted that water samples were used for this study. The analysis of water is considerably less problematic than that of the fish in the ponds because of sample collection, sample preparation and sensitivity of the analysis (Grimm, Lloyd & Zimba 2004). The organisms in the 2 L samples were sedimented by centrifugation and qualitatively examined microscopically for the presence of known off-flavour producing cyanobacteria.

GC–MS analysis

Geosmin and 2-MIB concentrations were determined using solid-phase micro-extraction (SPME) GC–MS according to Lloyd, Lea, Zimba and Grimm (1998). Geosmin, (9a, 10a-decalol; CAS#: 19700-21-1) was obtained from Givaudan (Clifton, NJ, USA), 2-MIB, ([1R-exo]-1,2,7,7-tetramethyl-[2,2,1]-bicyclo-heptan-2-ol; CAS#: 2371-42-8) and borneol ([1R]-endo-1,7,7-trimethyl bicyclo[2.2.1] heptan-2-ol; CAS#: 464-43-7) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Standards of 1 g mL^{-1} in neat ethanol were diluted with deionized water to obtain mg L^{-1} concentrations. Three grams of NaCl were added and the vial spiked with 5 μL of a 10 mg L^{-1} solution of the internal standard, decahydro-1-naphthol (50 ng). The vial was sealed with a crimp cap fitted with a Viton septum and placed in a CTC SPME autosampler (Leap Technologies, Carrboro, NC, USA). Samples were maintained at room temperature until analysed.

The sample was then heated to 65 °C and exposed to the SPME fibre for a 12 min adsorption period while undergoing vigorous agitation. The autosampler was equipped with a 1 cm long, divinylbenzene/carboxen/polydimethylsiloxane SPME fibre (Supelco, Bellefonte, PA, USA). The fibre was withdrawn from the sample and desorbed at 270 °C for 5 min in the injection port of an HP6890 GC equipped with a 5973 mass selective detector (Hewlett Packard, Palo Alto, CA, USA). The injection port was operated in pulsed splitless mode and fitted with a 0.7 mm ID injection liner. The head pressure was set to 25 psi of helium for the first minute, and then to a constant velocity of 40 cm s^{-1} for the remainder of the GC run. For quantitative analysis using selected ion monitoring (SIM), the oven was initially held at 80 °C for 1 min then ramped 20 °C min^{-1} to 100 °C, 7.5 °C min^{-1} to 152 °C, 65 °C min^{-1} to 250 °C, where the oven was held to give a total run time of 12.75 min. Cool down for the GC oven took approximately 4 min. To eliminate carryover between samples, after washing, glassware was rinsed with a 1 M HCl solution, followed by a water rinse and baked at 200 °C.

Weather data

Data was retrieved from the LSU Agriculture Center server located in Baton Rouge, LA (http://www.lsuagcenter.com/weather/). The remote weather station, Rice, reporting the weather conditions is located northeast of Crowley, LA (latitude 30°14′28″N, longitude 92°20′51″W, elevation 25 ft), 18 km from the catfish farm. The area surrounding the farm and the weather station is a mixture of open farm land and forested land. The LSU maintains the weather station that consists of instruments to collect the data and transmit it to the central server. The detection instruments are connected to a CR23X datalogger (Campbell Scientific, North Logan, UT, USA). A datalogger is a specialized computer that accepts electronic signals from various instruments, performs mathematical functions on the data, and records summaries in internal memory at designated intervals. Data were transmitted to a centralized computer every 5 min. Soil temperature data come from four Type T (copper–constantan) thermocouples at depths of 0 cm (just barely covered by dirt) and 5, 10 and 25 cm, in the centre of bare soil plots at least 8 ft^2. A dual sensor measures temperature and relative humidity using a HMP35A (Vasiala Ojy, Helsinki, Finland), equipped with a platinum temperature sensing element, and a Humicap relative humidity sensor. The station has a second temperature sensor, identical to that used in National Weather Service electronic Maximum Minimum Temperature Systems, used to judge the quality of the primary temperature sensor's data. Both wind speed and direction measurements are taken with an RM Young Wind Monitor (Traverse City, MI, USA), configured for use with Campbell Scientific dataloggers, at a height of 10 m. For precipitation, a tipping bucket rain gauge is employed. The unit transmits a signal to the datalogger each time 0.01 in of rainfall accumulates.

Statistical analysis

Descriptive statistics were computed for both continuous (weather measures and off-flavour compound concentrations) and categorical variables (limit of detection classification). For geosmin and 2-MIB, the limits of detection were set to 0.015 and 0.035 μg L^{-1} (Howgate 2004). To test for the presence of significant associations between variables, correlation coefficients were computed. For assessing the strength of the correlation coefficients, the interpretive guidelines by Cohen (1988) were followed: 0.10–0.29 small (weak), 0.30–0.49 medium (moderate), and 0.50–1.00 large (strong). As the main goal of the study was to determine if the weather variables could be used to predict a pond's flavour classification (on- or off-flavour; binary outcome), logistic regression models were built. Logistic regression models or logit models are used when the dependent variable (Y) has a dichotomous (binary; only two possible outcomes) form (Allison 1999). With these models, the dependent variable is actually expressed as a logit or log-odds. That is,

$$Y = \log \frac{P}{1-P}$$

where P is the probability that $Y = 1$ and log is the natural logarithm. The independent variables' coefficients resulting from these models have a simple interpretation in terms of odds ratios. Odds ratios are obtained by exponentiating the coefficients of the independent variables.

The dependent variable was the weekly flavour status of each pond where off-flavour status was considered the 'event.' The independent or predictor variables included weather measures (air and soil temperature, rainfall, wind velocity and humidity) and pond size. The logistic regression model has the following form:

$$Y_{ij} = \beta X_j + \gamma Z_i + \varepsilon_{ij}$$

where the observed outcome value (Y_{ij}) for the ith pond observed at the jth week is expressed as a function of a vector of continuous weather variables $\mathbf{X}_j$ measured at the jth week and a continuous size variable Z_i measured for the ith pond. Any differences between this predicted value and the observed value are attributed to the residual error ($\boldsymbol{\varepsilon}_{ij}$). Model selection was based upon significance of the explanatory variables and Akaike's information criteria (AIC). Results were considered significant at the nominal level of 0.05. All statistical analyses were performed using SAS® software version 9.1 (SAS Institute, Cary, NC, USA). The

SAS procedure PROC LOGISTIC was used to fit the logistic regression models for pond flavour status.

Results

The study included 44 weeks of data collection for a total of 21 ponds. However, for some ponds, data were not collected for the entire 44 weeks due to draining and maintenance (ponds 8 and 13). The dataset consisted of 888 pond-week values. The data for geosmin and 2-MIB concentrations in the water of 18 of the ponds are shown in Fig. 2. (Pond 36 data is not shown). The limits of detection by humans were taken from Howgate (2004) and represent an intermediate value of recently reported values (Grimm *et al*. 2004).

Mean minimum and maximum air temperatures were 15 and 26 °C, respectively, while mean minimum and maximum soil temperatures were 21 and 25 °C respectively (Table 1). Mean average wind velocity was 9.2 km h^{-1}; mean rainfall was 4.6 cm. Mean minimum and maximum humidity levels were 57% and 100% respectively. Descriptive statistics for the off-flavour compounds geosmin and 2-MIB can be found in Table 2. Mean weekly geosmin concentrations ranged from 0.004 to 0.246 mg L^{-1}, whereas mean weekly 2-MIB concentrations ranged from 0.010 to 3.438 mg L^{-1} for the 21 ponds. Due to the high degree of skewness present in the geosmin and 2-MIB distributions, Spearman correlation coefficients (ρ) were computed to check for significant correlations between the two variables as well as among the weather variables. As can be seen in Table 3,

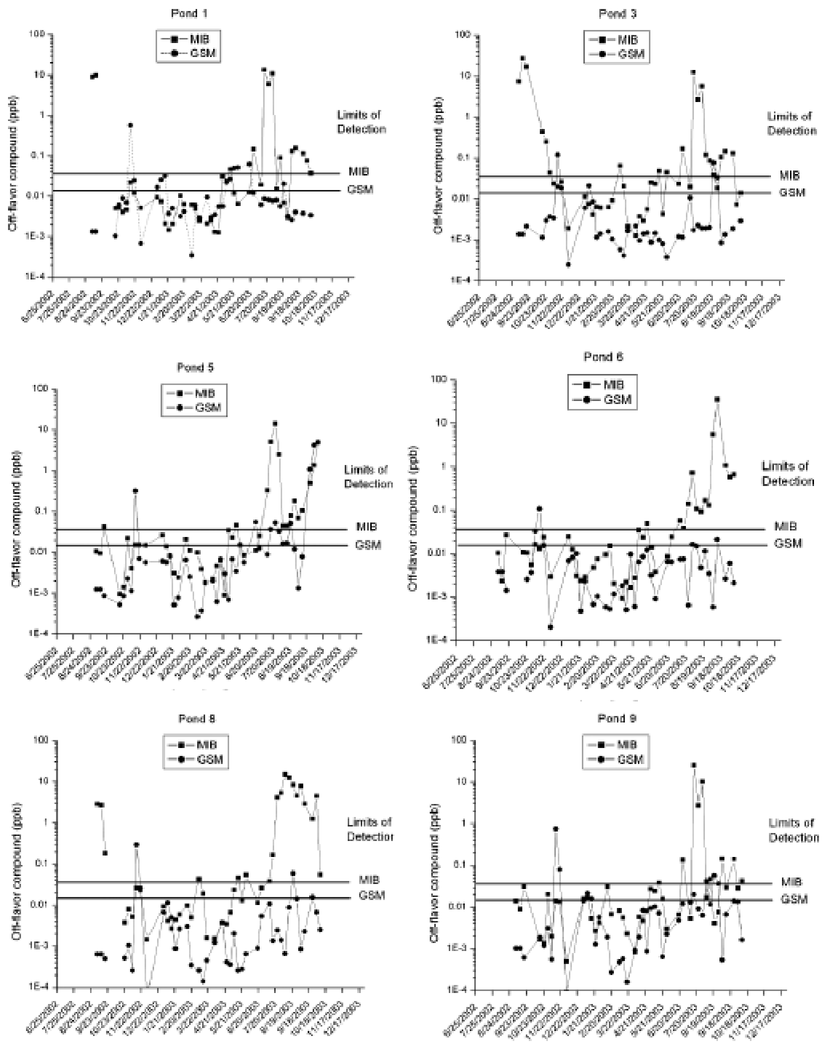


Figure 2 Data from gas chromatography–mass spectrometry determination of geosmin and 2-methylisoborneol levels. The limits of detection are indicated by horizontal bars. Data from 18 ponds are shown.

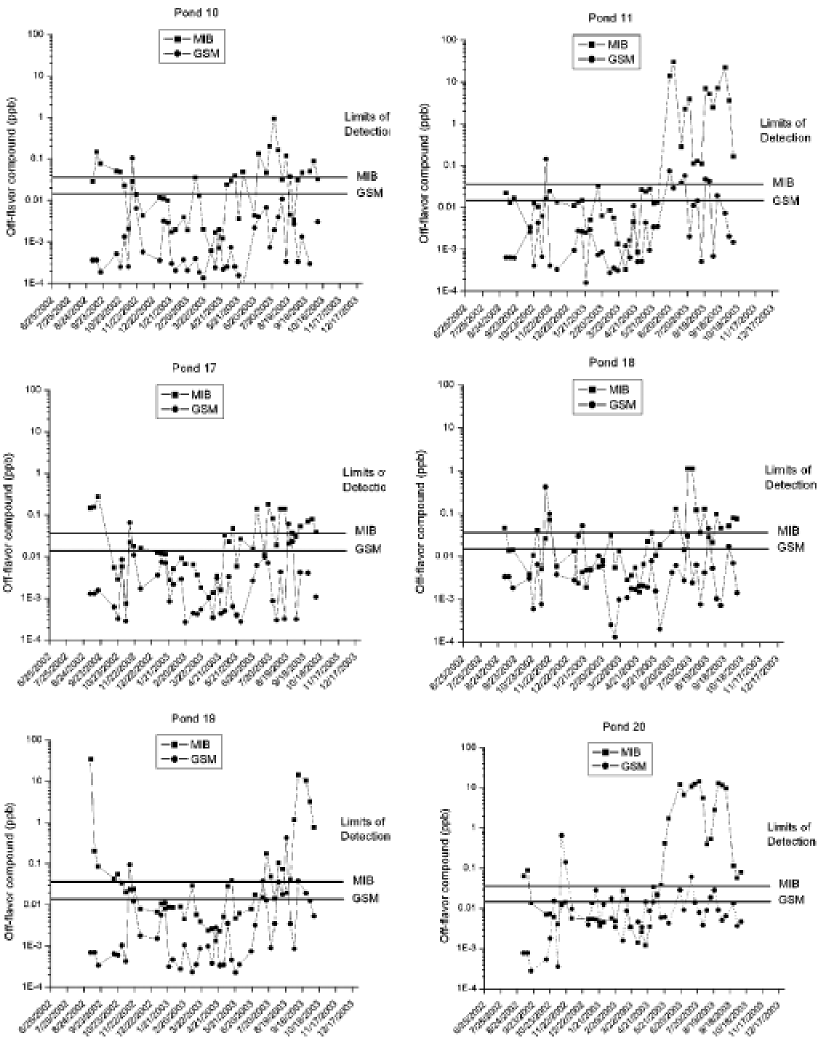
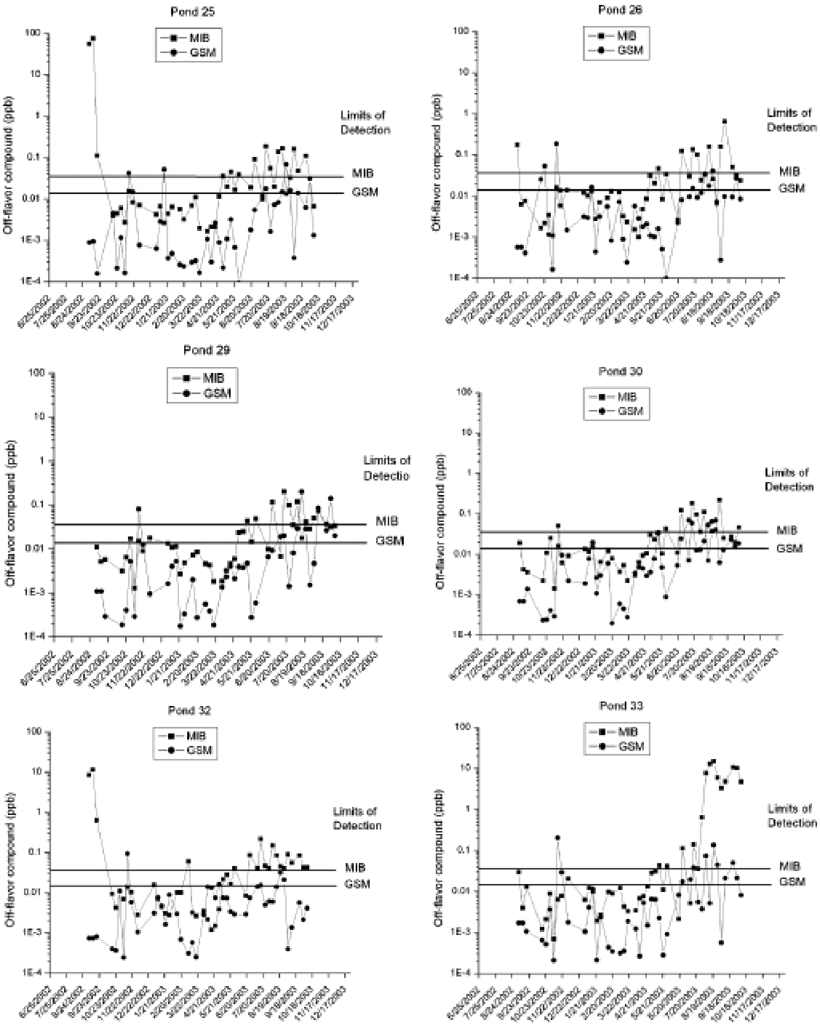


Figure 2 Continued.

there was a moderate correlation between geosmin and 2-MIB concentrations ($\rho = 0.2992$, $P < 0.0001$). Additionally, geosmin concentration was weakly correlated with air and soil temperatures (both minimum and maximum values), and had a weak inverse relationship with wind velocity. Essentially no bivariate relationships existed between geosmin and rainfall, maximum and minimum humidity, or pond size. 2-MIB concentration was strongly correlated with air and soil temperatures (both minimum and maximum values), moderately and inversely correlated with wind velocity, and weakly correlated with maximum humidity. Similar to geosmin, no bivariate relationships existed between 2-MIB and rainfall, minimum humidity or pond size. The percentages of pond-weeks in which geosmin and 2-MIB off-flavour occurred were 18.8% and 34.7% respectively.

The best fit for pond flavour status using the geosmin detection limit was provided by a model that included three explanatory variables – minimum soil temperature, rainfall and minimum humidity (Wald test statistic = 34.3, $P < 0.0001$, % concordant = 64.5). Estimates for the model coefficient standard errors, odds ratios and their corresponding 95% confidence intervals, and *P*-values may be found in Table 4. Minimum humidity had a protective effect on flavour status as shown by its estimated odds ratio of 0.96. This result indicates that for every 1% increase in minimum humidity there was a 4% [$(0.96 - 1) \times 100\%$] decrease in risk of a pond going off-flavour. Minimum soil temperature and rainfall were risk factors with odds ratios of 1.07 for both variables. These results indicate that for every 1 °C increase in soil temperature there was a 7% increase in risk of a pond



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going off-flavour, and for every additional cm of rainfall there was a 7% increase in risk.

The best fit for pond flavour status as determined by the 2-MIB detection limit was provided by a model that included two explanatory variables – minimum soil temperature and average wind velocity (Wald test statistic = 199.2, $P < 0.0001$, % concordant = 85.9). Estimates for the model coefficient standard errors, odds ratios and their corresponding 95% confidence intervals, and *P*-values may be found in Table 4. Minimum soil temperature was a risk factor for going off-flavour as shown by its estimated odds ratios of 1.35. This result indicates that for every 1 °C increase in minimum soil temperature, there was a 35% increase in risk of a pond going off-flavour. Average wind velocity had a protective effect on flavour status as shown by its estimated odds ratio of 0.85. This result

Table 1 Descriptive statistics for weather measures ($n = 44$ weeks)

Variable	Mean	Median	SD	Minimum	Maximum
Maximum air temperature (°C)	25.9	28.3	6.58	11.3	34.9
Minimum air temperature (°C)	15.4	16.2	7.02	1.7	23.8
Rainfall (cm daily)	4.6	4.2	4.13	0.0	21.5
Average wind velocity ($km\,h^{-1}$)	9.2	8.7	2.79	4.3	16.9
Maximum humidity (%)	99.7	100.0	1.16	93.1	100.0
Minimum humidity (%)	56.7	55.7	8.86	38.8	80.3
Maximum soil temperature (°C)	25.2	28.7	7.56	10.2	34.1
Minimum soil temperature (°C)	20.5	21.9	6.42	8.9	28.4

SD, standard deviation.

Table 2 Descriptive statistics for GSM and MIB by Pond

	GSM (ppb)						MIB (ppb)					
Pond	*n*	Mean	Median	SD	Minimum	Maximum	*n*	Mean	Median	SD	Minimum	Maximum
1	44	0.024	0.010	0.0855	0.000	0.570	44	1.151	0.010	3.2894	0.000	13.750
3	44	0.008	0.002	0.0214	0.000	0.120	44	1.681	0.023	5.1396	0.001	26.915
5	44	0.246	0.010	0.9599	0.000	4.870	44	0.668	0.010	2.3351	0.000	14.060
6	44	0.008	0.000	0.0174	0.000	0.110	44	1.018	0.010	5.3479	0.000	35.260
7	44	0.013	0.000	0.0268	0.000	0.160	44	3.438	0.030	6.0292	0.000	21.090
8	43	0.011	0.000	0.0449	0.000	0.290	43	1.689	0.020	3.4576	0.000	15.080
9	44	0.027	0.005	0.1128	0.000	0.750	44	0.891	0.010	4.0812	0.000	25.290
10	44	0.004	0.000	0.0176	0.000	0.110	44	0.057	0.020	0.1421	0.000	0.920
11	44	0.011	0.000	0.0264	0.000	0.140	44	2.231	0.015	5.9309	0.000	29.980
13	9	0.042	0.000	0.1267	0.000	0.380	9	0.010	0.010	0.0112	0.000	0.030
17	44	0.005	0.000	0.0141	0.000	0.070	44	0.044	0.020	0.0615	0.000	0.280
18	44	0.017	0.000	0.0633	0.000	0.410	44	0.080	0.015	0.2301	0.000	1.120
19	44	0.017	0.000	0.0644	0.000	0.420	44	1.484	0.020	5.7148	0.000	34.280
20	44	0.028	0.008	0.0990	0.000	0.653	44	2.333	0.024	4.4875	0.001	14.265
25	44	0.007	0.000	0.0147	0.000	0.070	44	3.007	0.010	13.9346	0.000	75.130
26	44	0.012	0.000	0.0285	0.000	0.180	44	0.045	0.010	0.1031	0.000	0.640
29	44	0.016	0.000	0.0390	0.000	0.200	44	0.028	0.010	0.0398	0.000	0.200
30	44	0.014	0.005	0.0205	0.000	0.070	44	0.031	0.010	0.0465	0.000	0.220
32	44	0.008	0.000	0.0146	0.000	0.090	44	0.494	0.020	2.0961	0.000	11.280
33	44	0.016	0.000	0.0370	0.000	0.200	44	1.725	0.010	3.7853	0.000	14.760
36	44	0.045	0.010	0.1211	0.000	0.740	44	1.080	0.045	3.4742	0.000	21.510

SD, standard deviation; GSM, geosmin; MIB, methylisoborneol.

Table 3 Spearman correlation coefficients (ρ) for bivariate relationships between off-flavour compounds and weather measures ($n = 888$ pond-weeks)

	GSM		MIB	
Variable	ρ	*P*-value	ρ	*P*-value
MIB	0.2992	<0.0001		
Pond size	−0.0069	0.8383	0.0591	0.0784
Maximum air temperature	0.2082	<0.0001	0.6171	<0.0001
Minimum air temperature	0.1805	<0.0001	0.6224	<0.0001
Rainfall	0.0702	0.0365	0.0819	0.0146
Average wind velocity	−0.1437	<0.0001	−0.4716	<0.0001
Maximum humidity	−0.0070	0.8353	0.1357	<0.0001
Minimum humidity	−0.0929	0.0056	0.0477	0.1554
Maximum soil temperature	0.2086	<0.0001	0.5837	<0.0001
Minimum soil temperature	0.2136	<0.0001	0.6262	<0.0001

GSM, geosmin; MIB, methylisoborneol.

indicates that for every 1 km h^{-1} increase in average wind velocity there was a 15% decrease in risk of a pond going off-flavour.

Discussion

In an attempt to understand the factors that affect off-flavour episodes in catfish production ponds, the concentrations of geosmin and 2-MIB were determined over a 44-week period and correlated with local weather parameters. Both geosmin and 2-MIB were positively correlated with air and soil temperatures, and negatively correlated with wind velocity. Additionally, 2-MIB was correlated with maximum humidity. The relationship between temperature and off-flavour episodes has been reported previously (Lelana 1987; Tucker & van der Ploeg 1993; van der

Table 4 Results of logistic regression models for off-flavour status by GSM and MIB limit of detection methods

Explanatory variable	Odds ratio	SE	95% CI	*P*-value
Off-flavour compound: GSM				
Minimum soil temperature	1.070	0.0151	1.039, 1.102	<0.0001
Minimum humidity	0.956	0.0123	0.933, 0.979	0.0003
Rainfall	1.071	0.0230	1.024, 1.121	0.0028
Off-flavour compound: MIB				
Minimum soil temperature	1.349	0.0255	1.284, 1.419	<0.0001
Average wind velocity	0.845	0.0362	0.787, 0.907	<0.0001

SE, standard error of model coefficient, 95% CI, 95% confidence interval for odds ratio.
GSM, geosmin; MIB, methylisoborneol.

Ploeg & Tucker 1994; Zimba *et al.* 2001; reviewed by Tucker 2000) and is commonly held knowledge among catfish farmers. Wind will concentrate cyanobacteria on or near the surface of the pond on the downwind side, increase degassing by turbulent mixing and thereby cause a corresponding change in the distribution of off-flavour compounds. There were no bivariate relationships between rainfall, minimum humidity or pond size and levels of either off-flavour compound. The lack of association between rainfall and the off-flavour compounds was somewhat surprising, as rain would serve to cool the air and soil temperatures, which do impact off-flavour levels. However, when rainfall was covaried with soil temperature and minimum humidity, it did exhibit a significant predictive ability for off-flavour status as determined by geosmin levels. While pond size effects have been reported by Zimba *et al.* (2001), the effect was only observed in ponds of approximately 3 ha or less. As the majority (86%) of the ponds in this study exceeded 3 ha in size, our results support those reported by Zimba *et al.* (2001). For geosmin levels, minimum soil temperature, minimum humidity and rainfall proved to be the best predictors of off-flavour status, correctly predicting an off-flavour event 64.5% of the time. For 2-MIB levels, the best predictors were minimum soil temperature and wind velocity, correctly predicting an off-flavour event 85.9% of the time. Hence, for off-flavour determined by either geosmin or 2-MIB, soil temperature plays a significant role as a risk factor. However, while humidity plays a protective role in off-flavour determined by geosmin, wind velocity is a protective factor for off-flavour determined by 2-MIB. The protective role of humidity is somewhat puzzling, as rainfall is a risk factor for off-flavour status. However, this protective effect is not believed to be due to a statistical artefact such as colinearity between humidity and rainfall that can lead to a reversal in odds ratios. While there was a moderate correlation between rainfall and minimum humidity (0.4281, $P = 0.0037$), only 18% of the variance in rainfall can be explained by the variance in minimum humidity. Hence, colinearity is not believed to have caused the protective effect of minimum humidity. Additionally, rainfall was a significant risk factor for off-flavour determined by geosmin, but had no effect on off-flavour determined by 2-MIB. Reasons for these differing predictors of off-flavour as determined by these two methods are unknown and deserve further study. One possible reason for these differences concerns the formation of these off-flavour compounds by different algal species (Smith, Boyer & Zimba 2008).

In consideration of this data, it is important to note that fish 'bio-concentrate' these off-flavour compounds. Reports on the level of bio-concentration vary from three-fold to four-fold (Grimm, pers. comm.) to ten-fold or more (Lelana 1987; Johnsen & Lloyd 1992). Bio-concentration is dependent on temperature of the water and the fat content of the fish (Johnsen, Lloyd, Vinyard & Dionigi 1996). Thus, even values below the limits of detection in water that were used in this analysis could render the fish off-flavour.

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